either "stealthing" the surface or decorating it to resemble the body's own cell membranes in order to insure that the carrier circulates long enough to reach its target.

Another disadvantage of phospholipid liposome based carriers is that many of the lipid components are isolated from plant or animal tissues. This can raise concerns as to the levels of contaminants, such as endotoxins, that might be present in the preparations.

The third disadvantage is that the phospholipid liposome membranes are fluid, i.e. lipid components can move around changing their spatial orientation toward one another. Alteration in the spatial relationship between presented antigens can give rise to particles that have reduced immunogenicity (Chackerian, et al., "Induction of Autoantibodies to Mouse CCR5 with Recombinant Papillomavirus Particles," *Proc. Natl. Acad. Sci. USA* 96(5):2373-2378 (1999)).

A fourth disadvantage to phospholipid based liposomes arises from their propensity to fuse to cell membranes or other administered lipid carriers that can result in an amalgamation and loss of specific particles, particle contents or particle size uniformity, and therefore, lead to ineffectiveness of a vaccine or therapeutic based on such materials.

Polymerization of lipid-based nanoparticles creates a stable structure that does not readily fuse with other polymerized liposome nanoparticles or cell membranes, and therefore these nanoparticle vaccine carriers can maintain their small and uniform size. Polymerized liposome nanoparticles have been described in various patent and journal publications. For example, U.S. Pat. No. 6,004,534 to Langer, et al.; Brayden, et al., "Microparticle Vaccine Approaches to Stimulate Mucosal Immunisation," *Microbes and Infection* 3(10):867-876 (2001); Clark, et al., "Targeting Polymerized Liposome Vaccine Carriers to Intestinal M Cells," *Vaccine* 20:208-217 (2002); and Chen, et al., "Lectin-bearing Polymerized Liposomes as Potential Oral Vaccine Carriers," *Pharm. Res.* 13(9):1378-1383 (1996), relate to targeted polymerized liposomes for oral and/or mucosal delivery of encapsulated material as vaccines, allergens and therapeutics. Jeong, et al., "Enhanced Adjuvantic Property of Polymerized Liposome as Compared to a Phospholipid Liposome," *J. Biotech.* 94:255-263 (2002), also describes encapsulation of materials in a polymerized liposome, which is non-targeted. These references all describe encapsulation of materials in phospholipid-based polymerized nanoparticles. The disadvantages of phospholipid-based carriers have been discussed above.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that nanoparticle vaccines having multivalent surface antigens (presented on the exterior or interior or the particle) or encapsulated antigens elicit significantly increased immune responses (Guan, et al., "Liposomal Formulations of Synthetic MUC1 Peptides: Effects of Encapsulation Versus Surface Display of Peptides on Immune Responses," *Bioconj. Chem.* 9:451458 (1998), which is hereby incorporated by reference in its entirety). Additionally, co-display of targeting molecule(s) on the polymerized liposome nanoparticle for purposes of directing the vaccine to a specific in vivo location increases the efficiency and effectiveness of the desired immune response.

Polymerization of the membrane greatly "freezes" the positions of the items displayed on the particle surface. As presentation of antigenic elements in a polyvalent array is believed to be an important contributor toward promoting an immunological response (Chackerian, et al., "Induction of Autoantibodies to Mouse CCR5 with Recombinant Papillomavirus Particles," *Proc. Natl. Acad. Sci. USA* 96(5):2373-2378 (1999), which is hereby incorporated by reference in its entirety), a fixed surface-displayed rigid array is likely to be a more successful antigenic presenter than a fluid surface. Once the polymerized particle is prepared and assayed for vaccine effectiveness surface changes which may alter its activity or toxicity are unlikely to occur.

In the present invention, antigens may also be contained inside the nanoparticle, with or without surface displayed antigens and/or targeting molecules, depending upon the specific disease application. The present invention provides compositions and methods for use in various pharmaceutical applications, including vaccinating a subject for protection against infection by a pathogenic agent or for vaccination of a subject for resolution of a chronic infectious disease. Such subjects may include humans and wild or domestic animal populations such as bison, elk, cows, horses, sheep, goats, pigs, fowl, cats and dogs, although this invention may be applied to other species as well. Administration of the vaccine of this invention may be carried out orally, intradermally, intermuscularly, intraperitoneally, intravenously, subcutaneously, intranasally, sublingually, buccally, vaginally, or rectally.

In a preferred embodiment, the present invention relates to a nanoparticle that comprises a carrier, and polymerized liposome carriers are preferred, although various other carriers known to persons skilled in the art also would be appropriate. The polymerized liposome carrier may be either phospholipid or non-phospholipid based. The carrier preferably carries or displays (on the interior or exterior) multiple copies of antigen or combination of different antigens and targeting molecules. In another preferred embodiment the antigen-displaying carrier does not include targeting molecule(s). In a third preferred embodiment, the carrier displays antigen or a combination of different antigens and a targeting molecule on its surface, and encapsulates antigen or a combination of antigens within the nanoparticle. In another preferred embodiment, the antigen-displaying carrier encapsulates antigen(s) but does not display targeting molecule(s). In yet another preferred embodiment, the carrier displays targeting molecule(s) without antigen on its surface and encapsulates antigen or a combination of antigens within the nanoparticle.

According to the methods and compositions of the present invention, surface exposed antigen(s) and/or targeting molecule(s) may be attached to the nanoparticles by any means known in the art. Conjugation methods of this invention include chemical complexing, which may be either ionic or non-ionic in nature, or covalent binding. Such conjugation of antigen or targeting molecule may occur to reactive head groups of individual lipid monomers, or a collection of lipid monomers prior to assembly of the nanoparticle. Alternatively the antigen or targeting molecule can be attached to reactive head groups after the polymerized nanoparticle is formed.

The antigen or antigens of the present invention that are displayed on or within the nanoparticle induce an immune response against onset of disease caused by a variety of pathogenic conditions. In a preferred embodiment, the antigen may be derived from, but are not limited to, pathogenic bacterial, fungal, or viral organisms, *Streptococcus* species, *Candida* species, *Brucella* species, *Salmonella* species, *Shigella* species, *Pseudomonas* species, *Bordetella* species, *Clostridium* species, Norwalk virus, *Bacillus anthracis, Mycobacterium tuberculosis*, human immunodeficiency virus (UV), *Chlamydia* species, human Papillomaviruses,